HORTSCIENCE 54(11):2056–2062. 2019. https://doi.org/10.21273/HORTSCI14342-19

Salt Tolerance of Sego Supreme™ Plants

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Additional index words. *Aquilegia barnebyi*, *Clematis fruticosa*, *Epilobium septentrionale*, mineral nutrient, *Tetraneuris acaulis*, visual score

***Abstract*. Sego Supreme™ is a designated plant breeding and introduction program at the Utah State University Botanical Center and the Center for Water Efficient Landscaping. This plant selection program introduces native and adapted plants to the arid West for aesthetic landscaping and water conservation. The plants are evaluated for characteristics such as color, flowering, ease of propagation, market demand, disease/pest resistance, and drought tolerance. However, salt tolerance has not been considered during the evaluation processes. Four Sego Supreme™ plants [*Aquilegia barnebyi* (oil shale columbine), *Clematis fruticosa* (Mongolian gold clematis), *Epilobium septentrionale* (northern willowherb), and *Tetraneuris acaulis* var. *arizonica* (Arizona four-nerve daisy)] were evaluated for salt tolerance in a greenhouse. Uniform plants were irrigated weekly with a nutrient solution at an electrical conductivity (EC) of 1.25 dS·m^{-1} as control or a saline solution at an EC of 2.5, 5.0, 7.5, or 10.0 dS·m^{-1} for 8 weeks. After 8 weeks of irrigation, *A. barnebyi* irrigated with saline solution at an EC of 5.0 dS·m^{-1} had slight foliar salt damage with an average visual score of 3.7 (0 = dead; 5 = excellent), and more than 50% of the plants were dead when irrigated with saline solutions at an EC of 7.5 and 10.0 dS·m^{-1}. However, *C. fruticosa, E. septentrionale*, and *T. acaulis* had no or minimal foliar salt damage with visual scores of 4.2, 4.1, and 4.3, respectively, when irrigated with saline solution at an EC of 10.0 dS·m^{-1}. As the salinity levels of treatment solutions increased, plant height, leaf area, and shoot dry weight of *C. fruticosa* and *T. acaulis* decreased linearly; plant height of *A. barnebyi* and *E. septentrionale* also declined linearly, but their leaf area and shoot dry weight decreased quadratically. Compared with the control, the shoot dry weights of *A. barnebyi*, *C. fruticosa*, *E. septentrionale*, and *T. acaulis* decreased by 71.3%, 56.3%, 69.7%, and 48.1%, respectively, when irrigated with saline solution at an EC of 10.0 dS·m^{-1}. *Aquilegia barnebyi* and *C. fruticosa* did not bloom during the experiment at all treatments. Elevated salinity reduced the number of flowers in *E. septentrionale* and *T. acaulis*. Elevated salinity also reduced the number of shoots in all four species. Among the four species, sodium (Na^+) and chloride (Cl^-) concentration increased the most in *A. barnebyi* by 53 and 48 times, respectively, when irrigated with saline solution at an EC of 10.0 dS·m^{-1}. In this study, *C. fruticosa* and *T. acaulis* had minimal foliar salt damage and less reduction in shoot dry weight, indicating that they are more tolerant to salinity. *Epilobium septentrionale* was moderately tolerant to saline solution irrigation with less foliar damage, although it had more reduction in shoot dry weight. On the other hand, *A. barnebyi* was the least tolerant with severe foliar damage, more reduction in shoot dry weight, and a greater concentration of Na^+ and Cl^-.**

Field trials are used to successfully identify suitable plants with outstanding landscape performance for various plant selection and evaluation programs. Superior plants are selected mostly on the basis of their appearance, environmental adaptability, drought tolerance, and disease/pest tolerance. For example, Earth-Kind® is one of the special plant selection programs of the Texas A&M Agrilife Extension Service (Aggie Horticulture, 2019). A total of 21 garden roses (*Rosa* ×*hybrida*) with superior stress tolerance and outstanding landscape performance were categorized as Earth-Kind® Roses. Similarly, Texas Superstar® program promotes superior plants that can grow well at various locations in Texas with minimal soil preparation and irrigation and no pesticides (Mackay et al., 2001). Sixteen perennial ornamental plants have been designated as Texas Superstar® perennials. Colorado State University has collaborated with the Denver Botanic Gardens to establish the Plant Select® program to promote plants annually which are water-wise and adapted to the Rocky Mountains (Colorado Gardening, 2019). Native plants from arid and semiarid environments, introduced by Plant Select® program, are excellent candidates for water-efficient landscaping. Sales of native plants have grown from $1.46 million in 2007 to $1.68 million in 2012 through Plant Select® program (National Information Management and Support System, 2019). However, these programs have overlooked the salinity tolerance of the plants during the selection and evaluation processes.

Sego Supreme™ is a plant breeding and introduction program developed by Utah State University (USU) Botanical Center and the USU Center for Water-Efficient Landscaping with the intention of introducing native and adaptable plants into arid west landscapes to conserve water without compromising the aesthetic value of the landscapes (Anderson et al., 2014). Sego Supreme™ plants are evaluated for characteristics such as color, flowering, ease of propagation, market demand, disease/pest resistance, and drought tolerance. However, salinity tolerance has not been considered during the evaluation processes.

Soil salinity is one of the major obstacles for horticultural production all over the world. Soil is defined as a saline soil when the salinity level in a plant root zone exceeds 4 dS·m^{-1}, which affects plant growth and may not be suitable to grow plants (Chinnusamy et al., 2005). In many parts of the world, poor-quality water (such as reclaimed water that contains high concentrations of soluble ions) is used to irrigate ornamental plants to conserve potable water (Cassaniti et al., 2013; Niu and Cabrera, 2010). Both saline soil and irrigation water can have adverse effects on plant performance by affecting nutrient availability and competitive uptake, transport, and partitioning within the plant (Grattan and Grieve, 1999). Plant species or cultivars have different responses to salinity (Munns and Tester, 2008; Niu and Cabrera, 2010). Therefore, selection and identification of salt-tolerant ornamental plants are crucial for nursery production and landscape use.

Plants selected from the Earth-Kind® and Texas Superstar® programs have been studied for salinity tolerance by independent researchers. The salt tolerance of 18 Earth-Kind® Rose cultivars was investigated, and it was concluded that 'Belinda's Dream', 'Climbing Pinkie', 'Mrs. Dudley Cross', 'Reve d'Or', and 'Sea Foam' roses were salt-tolerant when irrigated with saline water at an EC of 10.0 dS·m^{-1} (Cai et al., 2014). Sun et al. (2015) reported that Texas Superstar® perennials such as *Malvaviscus arboreus* var. *drummondii* (Turk's cap), *Ruellia brittoniana* 'Katie Blue' ('Katie Blue' ruellia), *Salvia farinacea* 'Henry Duelberg' ('Henry Duelberg' salvia), and *Verbena* ×*hybrida* 'Blue princess' ('Blue Princess' verbena) were tolerant to salinity levels at ECs of 5.0 and 10.0 dS·m^{-1}, whereas *Phlox paniculata* 'John Fanick' ('John Fanick' phlox), *Phlox paniculata* 'Texas Pink' ('Texas Pink' phlox), and *Salvia leucantha* (Mexican bush sage) were sensitive to salinity levels at ECs of 5.0 and 10.0 dS·m^{-1}.

Aquilegia barnebyi, *Clematis fruticosa*, *Epilobium septentrionale*, and *Tetraneuris acaulis* var. *arizonica* are categorized as Sego Supreme™ selections. *Aquilegia barnebyi* is a perennial plant that usually occurs on oil shale substrates and is native to northeastern Utah and adjacent parts of Colorado (U.S. Department of Agriculture, 2019). It is a drought-tolerant species and suitable for xeriscaping (Dave's Garden, 2019). *Clematis fruticosa* is an erect, woody shrub with insect and disease tolerance (Missouri Botanical Garden, 2019). It grows in medium moisture, well-drained soil and has some drought tolerance. *Epilobium septentrionale*, a drought-tolerant perennial plant native to California, can grow in thin patches of soil between rocks (California Flora Nursery, 2019). *Tetraneuris acaulis* var. *arizonica* is a perennial plant that can tolerate many soil types and does not need much water once established in the landscape (Moosa Creek Nursery, 2019). It is native to western United States from Idaho to New Mexico (U.S. Department of Agriculture, 2019). These plants have potential for adoption by the landscape industry, but their salinity tolerance is unclear. In this study, the four Sego Supreme™ plant species were irrigated with saline solution at different salinity levels in a greenhouse to determine their salinity tolerance through measuring their growth responses and mineral nutrient status.

Materials and Methods

Plant materials and culture. The study was conducted in a research greenhouse at USU in Logan, UT (lat. 41°45′28″N, long. 111°48′48″W, elevation 1409 m). On 24 Aug. 2018, 1-year-old Sego Supreme™ plants produced from cuttings were received in a square pot (10.5 × 10.5 × 12.3 cm) from the USU Botanical Center (Kaysville, UT). On 27 Aug. 2018, plants were transplanted into 3.8-L injection-molded, polypropylene container (PC1D-4; Nursery Supplies, Orange, CA) filled with a soilless growing substrate consisting of 75% peatmoss (Canadian sphagnum peatmoss; SunGro Horticulture, Agawam, MA), 25% vermiculite (Therm-O-Rock West, Chandler, AZ), and 24.3 g·ft^{-3} white athletic field marking gypsum (92% calcium sulfate dihydrate, 21% calcium, 17% sulfur; Western Mining and Minerals, Bakersfield, CA). All plants were watered with tap water (EC = 0.344 dS·m^{-1}; pH = 7.65). During the experiment, aphids (*Aphidoidea*) and whiteflies (*Aleyrodidae*) were observed on *C. fruticosa*. To control aphids and whiteflies, all plants were sprayed with abamectin (Avid® 0.15 EC; Syngenta Crop Protection, Greensboro, NC) at a rate of 0.1 mL per gallon as needed. During the experimental period, the average air temperature in the greenhouse was 24.9 ± 1.2 °C during the day and 22.0 ± 2.4 °C at night. The average daily light integral in the greenhouse was 24.8 ± 12.5 mol·m^{-2}·d^{-1}. Supplemental light at 160.4 μmol·m^{-2}·s^{-1} was provided using 1000-W high-pressure sodium lamps (Hydrofarm, Petaluma, CA) from 600 to 2200 HR when light intensity inside the greenhouse was less than 544 μmol·m^{-2}·d^{-1} from 10 Oct. to 14 Dec. 2018. Supplemental light was measured using a full-spectrum quantum meter (MQ-500; Apogee Instrument, Logan, UT).

Treatments. Plants were pruned to uniform height (≈13 cm), and flowers were removed before the experiment was initiated. From 15 Oct. to 3 Dec. 2018, treatment solutions were applied on a weekly basis up to 8 weeks. Plants were irrigated with 1 L of nutrient solution (control) at an EC of 1.25 dS·m^{-1} or saline solution at an EC of 2.5, 5.0, 7.5, or 10.0 dS·m^{-1} (Table 1) with a leaching fraction of 15.1% ± 6.4%. In addition, plants were watered with 500 mL of nutrient solution whenever the substrate surface became dry. The nutrient solution was prepared in a 100-L tank by adding 0.8 g·L^{-1} of 15N–2.2P–12.5K water-soluble fertilizer (Peters Excel 15–5–15 Cal-Mag Special; ICL Specialty Fertilizers, Dublin, OH) to tap water and used as a control. The saline solution was prepared with an addition of sodium chloride (NaCl; Fisher Chemical, Logan, UT) and calcium chloride ($CaCl_2$; Fisher Chemical, Logan, UT) at a molar ratio of 2:1 in the nutrient solution. The ECs of the solutions, measured using an EC meter (LAQUA Twin; Horiba, Kyoto, Japan), were 1.2 ± 0.1 (control, nutrient solution), 2.5 ± 0.2 (EC 2.5), 4.7 ± 0.1 (EC 5), 7.5 ± 0.2 (EC 7.5), and 9.8 ± 0.2 dS·m^{-1} (EC 10) (mean ± SD) during the experiment. The pH of the solutions was adjusted to 6.7 ± 0.4 using 1 M nitric acid.

Data collection. Leachate solution was collected during each application of treatment solutions. The EC of the leachate solution was determined on one plant per species per treatment using the pour-through method described by Cavins et al. (2008). Plant heights, the length of the longest shoot, were measured at the start and end of the experiment. A visual score of 0 to 5 was assigned to each plant where 0 = dead, 1 = severe foliar damage (>90% leaves with burn, necrosis, and discoloration), 2 = moderate foliar damage (90% to 50%), 3 = slight foliar damage (50% to 10%), 4 = minimal foliar damage (<10%), and 5 = excellent without foliar damage (Sun et al., 2015). Plant size was not considered while assigning the visual score. Leaf greenness of each plant was measured 4 d before harvest using a handheld chlorophyll meter [soil-plant analysis development (SPAD) 502 Plus; Minolta Camera Co., Osaka, Japan]. Four fully expanded leaves from each plant were chosen for the measurements. The number of flowers of *E. septentrionale* and *T. acaulis* were counted, whereas *A. barnebyi* and *C. fruticosa* did not flower during the experimental period. The number of shoots were counted, and leaf area was measured using an area meter (LI-3100; LI-COR® Biosciences, Lincoln, NE). In addition, shoot dry weight was taken after plants were dried in an oven at 70 °C for 3 d.

Table 1. The chemical compositions of tap water, nutrient solution (control), and saline solutions used in the experiment.[z]

Item[y]	Tap[x]	EC (dS·m^{-1}) 1.25	2.5	5.0[x]	7.5	10.0[x]
NaCl (g·L^{-1})	—	—	0.31	0.92	1.72	2.27
$CaCl_2$ (g·L^{-1})	—	—	0.30	0.88	1.65	2.18
Ca^{2+} (mg·L^{-1})	47.2	82.5	187.7	362.5	608.9	759.8
Mg^{2+} (mg·L^{-1})	17.3	27.6	31.6	29.1	26.0	30.9
Na^{+} (mg·L^{-1})	1.4	0.4	121.2	359.6	639.4	872.4
SO_4^{2-} (mg·L^{-1})	8.9	11.2	10.4	11.2	11.0	11.0
Cl^{-} (mg·L^{-1})	3.4	5.7	377.0	1,050	2,220	2,780
B (mg·L^{-1})	0	0.23	0.17	0.22	0.22	0.2
Sodium adsorption ratio (SAR)	0.04	0.07	2.15	4.88	6.88	8.42
Adjusted SAR	0.09	0.13	4.54	11.2	16.6	20.9

[z]A nutrient solution at an electrical conductivity (EC) of 1.25 dS·m^{-1} was prepared by adding 0.8 g·L^{-1} 15 N–2.2 P–12.5 K water-soluble fertilizer (Peters Excel 15–5–15 Cal-Mag Special) to tap water. Sodium chloride (NaCl) and calcium chloride ($CaCl_2$) were added to the nutrient solution to make saline solutions at an EC of 2.5, 5.0, 7.5, and 10.0 dS·m^{-1}.

[y]Calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), sulphate (SO_4^{2-}), chloride (Cl^{-}), and boron (B) ions.

[x]Adapted from Wang et al. (2019).

Received for publication 9 July 2019. Accepted for publication 26 Aug. 2019.

This research was supported in part by the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture Hatch project UTA01381, New Faculty Start-Up Funds from the Office of Research and Graduate Studies, the Center for Water-Efficient Landscaping at Utah State University. This research was also supported by the Utah Agricultural Experiment Station, Utah State University, and approved as journal paper no. 9226. We are grateful for valuable comments from Dr. Larry Rupp and other anonymous reviewers. We appreciate the financial support from Grassology Peak Discipline Foundation of Xinjiang Uygur Autonomous Region and National Modern Agricultural Technology & Industry System (CARS-34) of China to Yuxiang Wang as a visiting scholar at Utah State University, Logan, UT.

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Mineral analyses. Three Sego Supreme™ plants per species per treatment were selected randomly and ground with a stainless Wiley mill (Thomas Scientific, Swedesboro, NJ) and allowed to pass through a 1-mm mesh screen. Powder samples were sent to the USU Analytical Laboratories for mineral analyses. In brief, the powder samples were extracted using 2% acetic acid (Fisher Scientific, Fair Lawn, NJ) following the protocol described in Miller et al. (2013). The Cl^- concentration was determined using a flow injection analysis and ion chromatography system (QuikChem 8000; Lachat Instrument, Loveland, CO) and reported on a dry plant basis (mg·g^{-1}). For Na^+, calcium (Ca^{2+}), and potassium (K^+), 0.5 g of powder samples and 8 mL of nitric acid (HNO_3) were added into a digestion tube that was then placed in a digestion block (Environmental Express, Charleston, SC) at 95 °C for 1 h and subsequently cooled for 15 to 20 min. A total of 4 mL of 30% hydrogen peroxide (H_2O_2) was added into the digestion tube that was placed again in the digestion block at 95 °C for 30 min. The H_2O_2 addition was repeated two more times, and the tube was cooled for 15 to 20 min between H_2O_2 additions. Then the digestion tube was cooled at a room temperature, and deionized water was added to bring the final volume up to 25 mL. The digest was analyzed using an inductively coupled plasma-optical emission spectrometry (iCAP 6300 ICP-AES; Thermo Scientific, Waltham, MA) and reported on a dry plant basis (mg·g^{-1}).

Experimental design and data analyses. The experiment was conducted using a randomized complete block design with six blocks. Each block consisted of 20 plants (four species and five treatments). A two-way analysis of variance was conducted to test the effects of salinity and species on plant growth and mineral nutrient data. Linear and quadratic trend analysis was performed for all data. Correlation analysis was also carried out between mineral concentration and visual quality. All statistical analyses were performed using a generalized linear model in JMP (Version 13.2; SAS Institute, Cary, NC).

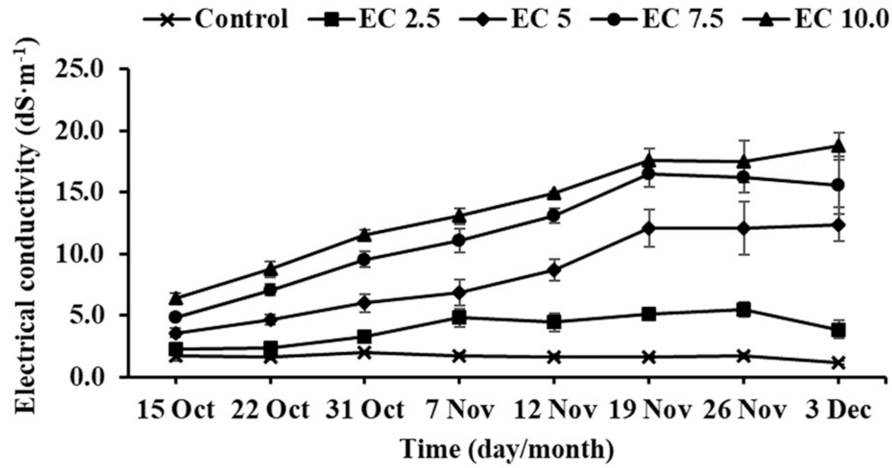

Fig. 1. Electrical conductivity (EC) of leachate solution collected after irrigating the Sego Supreme™ plants with a nutrient solution at an EC of 1.25 dS·m^{-1} or a saline solution at an EC of 2.5, 5.0, 7.5, or 10.0 dS·m^{-1}. A nutrient solution at an EC of 1.25 dS·m^{-1} was prepared by adding 0.8 g·L^{-1} 15 N–2.2P–12.5K water-soluble fertilizer (Peters Excel 15–5–15 Cal-Mag Special) to tap water. Sodium chloride (NaCl) and calcium chloride ($CaCl_2$) were added to the nutrient solution to make the saline solutions. Vertical bars represent standard errors of four measurements, one plant per treatment per species.

Results and Discussion

Leachate and substrate EC. As Sego Supreme™ plants were irrigated with saline solution, the salinity levels of leachate solution gradually increased (Fig. 1). When irrigated with saline solution at an EC of 2.5, 5.0, 7.5, or 10.0 dS·m^{-1}, the ECs of the leachate solutions increased from 2.3 to 5.5 dS·m^{-1}, 3.6 to 12.4 dS·m^{-1}, 4.9 to 16.5 dS·m^{-1}, and 6.4 to 18.8 dS·m^{-1}, respectively. These values were greater than the EC of the leachate solution (1.7 ± 0.2 dS·m^{-1}) when the nutrient solution was used. Leachate solution EC increased over time with increasing salinity of irrigation water, which verifies that salts accumulate in plant rhizosphere (Wu et al., 2016a).

Visual quality. Saline solution had different effects on the visual quality of the four Sego Supreme™ plant species (Fig. 2A). The visual score of *A. barnebyi* and *E. septentrionale* decreased linearly [r^2 = 0.95 (P < 0.0001) and r^2 = 0.94 (P < 0.0001), respectively] with increasing EC levels in the treatment solution. However, the visual score of *C. fruticosa* and *T. acaulis* decreased quadratically [R^2 = 0.998 (P = 0.008) and R^2 = 0.995 (P = 0.0002), respectively] as EC levels increased. *Aquilegia barnebyi* showed slight foliar salt damage when irrigated with saline solution at an EC of 5.0 dS·m^{-1} with an average visual score of 3.7, but more than 50% of plants died when irrigated with saline solution at an EC of 7.5 or 10.0 dS·m^{-1}. Similarly, *Aquilegia canadensis* (eastern red columbine) plants had moderate foliar salt damage when irrigated weekly with saline solution at an EC of 5.0 dS·m^{-1} for 8 weeks, and all plants died at an EC of 10.0 dS·m^{-1} (Wu et al., 2016a). Gerber et al. (2011) also reported that *Aquilegia* × *cultorum* 'Crimson Star' plants started to show foliar damage (marginal necrosis, chlorosis, and purpling) at the second weeks when they were watered with 0.05, 0.15, and 0.25 M NaCl solution (corresponding to about EC of 4.6, 11.0, and 18.3 dS·m^{-1}, respectively). *Clematis fruticosa*, *E. septentrionale*, and *T. acaulis* irrigated with saline solution at an EC of 10.0 dS·m^{-1} had no or minimal foliar salt damage with averaged visual score of 4.2, 4.1, and 4.3, respectively. These results demonstrated that *C. fruticosa*, *E. septentrionale*, and *T. acaulis* had a strong tolerance to the salinity levels tested in this study. Likewise, in a 3-year field study, it is reported that all *Clematis ispahanica* plants survived without any foliar damage and their visual quality was still acceptable up to an EC of 16.3 dS·m^{-1} in the treatment solution (Razmjoo and Aslani, 2018). Conversely, Wu et al. (2016b) reported that *Tetraneuris scaposa* (four-nerve daisy) showed moderate to severe foliar salt damage when irrigated with saline solution at an EC of 5.0 dS·m^{-1} for 5 weeks and were almost all dead at an EC of 10.0 dS·m^{-1}.

Plant height. Height of *A. barnebyi*, *C. fruticosa*, *E. septentrionale*, and *T. acaulis* decreased linearly [r^2 = 0.96 (P < 0.0001), r^2 = 0.94 (P = 0.0001), r^2 = 0.98 (P < 0.0001), and r^2 = 0.98 (P < 0.0001), respectively] as EC levels increased (Fig. 2B). Height of the four Sego Supreme™ plants irrigated with saline solution at an EC of 2.5 dS·m^{-1} were similar to that of the control. Saline solution at an EC of 5.0 dS·m^{-1} also did not affect the growth of *C. fruticosa*, but plants irrigated with saline solutions at an EC of 7.5 and 10.0 dS·m^{-1} were 13.4% and 19.6% shorter than the control, respectively. When plants were irrigated with saline solutions at an EC of 5.0, 7.5, and 10.0 dS·m^{-1}, plants were 36.7% to 63.7% shorter for *A. barnebyi*, 12.6% to 35.4% shorter for *E. septentrionale*, and 18.0% to 33.6% shorter for *T. acaulis*, respectively, compared with the respective control. Similarly, Wu et al. (2016a) observed a 75% reduction in a growth index (average of height and two perpendicular crown diameter) of *A. canadensis* when they were irrigated weekly with saline solution at an EC of 10.0 dS·m^{-1} for 8 weeks. Razmjoo and Aslani (2018) also found a 15% reduction in height of *C. ispahanica* when irrigated with saline solution at an EC of 16.3 dS·m^{-1}. In a 4-week study by Hadi et al. (2014), there was a significant reduction in growth of *Epilobium laxum* (lax willowherb) at 6000 ppm NaCl solution (EC of ≈7.5 dS·m^{-1}). *Tetraneuris scaposa* also had a 37.2% reduction in plant height when they were irrigated weekly with saline solution at an EC of 10.0 dS·m^{-1} for 5 weeks (Wu et al., 2016b).

Leaf area. Leaf area decreased linearly for *C. fruticosa* and *T. acaulis* [r^2 = 0.98 (P < 0.0001), and r^2 = 0.91 (P < 0.0001), respectively], but quadratically for *A. barnebyi* and *E. septentrionale* [R^2 = 0.98 (P = 0.0005)

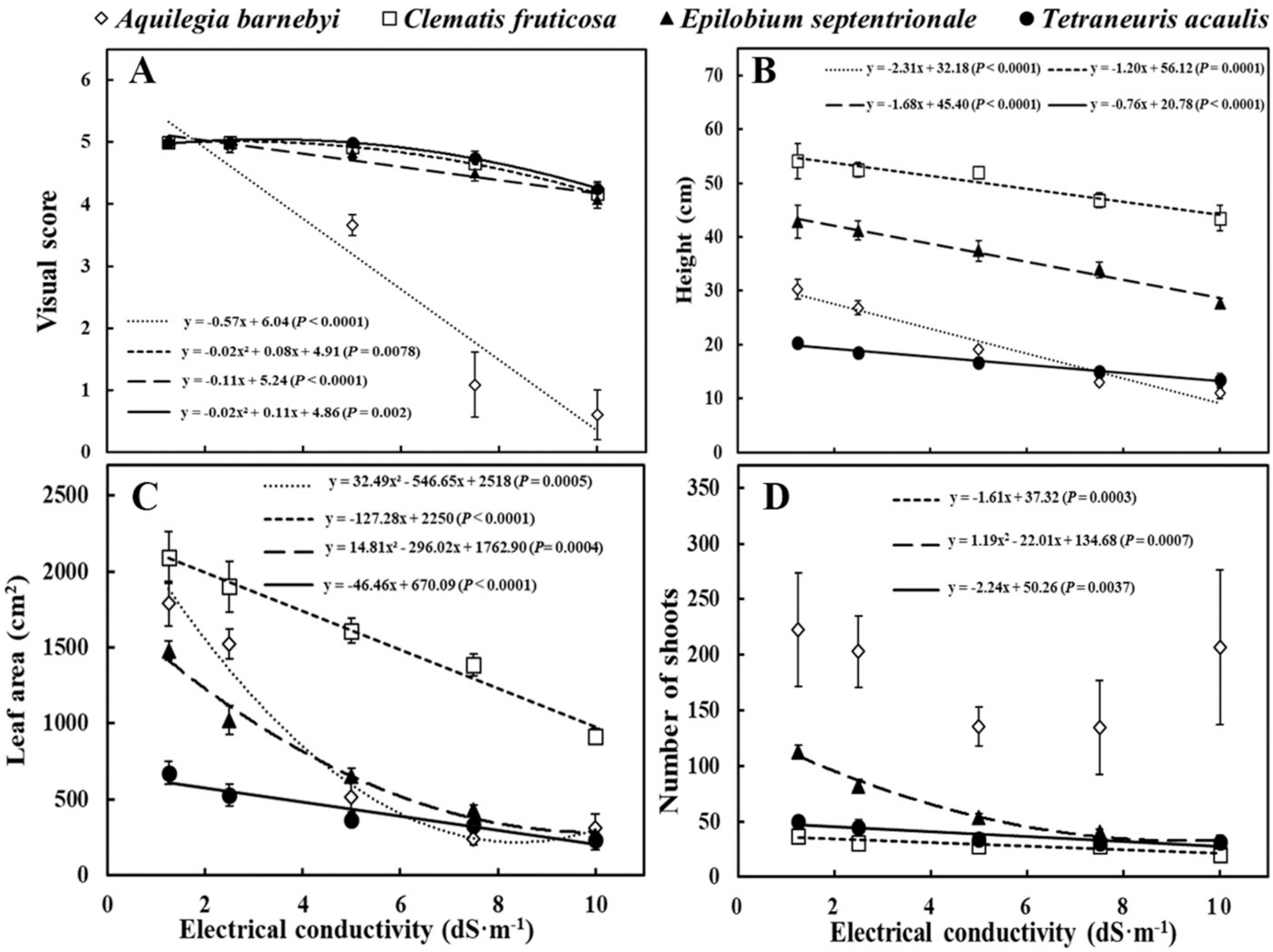

Fig. 2. Visual score (**A**), height (**B**), leaf area (**C**), and number of shoots (**D**) of Sego Supreme™ plants after irrigating a nutrient solution at an EC of 1.25 dS·m^{-1} or a saline solution at an EC of 2.5, 5.0, 7.5, or 10.0 dS·m^{-1}. Visual score reference scale: 0 = dead; 1 = severe foliar damage (>90% leaves with burn, necrosis, and discoloration); 2 = moderate foliar damage (50% to 90%); 3 = slight foliar damage (50% to 10%); 4 = good quality with minimal foliar damage (<10%); 5 = excellent without foliar damage. A nutrient solution at an EC of 1.25 dS·m^{-1} was prepared by adding 0.8 g·L^{-1} 15 N–2.2P–12.5K water-soluble fertilizer (Peters Excel 15–5–15 Cal-Mag Special) to tap water. Sodium chloride (NaCl) and calcium chloride ($CaCl_2$) were added to the nutrient solution to make the saline solutions. Vertical bars represent standard errors of six measurements.

and R^2 = 0.98 (P = 0.0004), respectively] with increasing EC levels (Fig. 2C). Compared with the control, the leaf area of *A. barnebyi*, *C. fruticosa*, *E. septentrionale*, and *T. acaulis* decreased by 82.5%, 56.2%, 82.5%, and 65.1%, respectively, when irrigated with saline solution at an EC of 10.0 dS·m^{-1}. Even at an EC of 5.0 dS·m^{-1}, *A. barnebyi* and *E. septentrionale* had a high reduction in leaf area of 71.2% and 55.7%, respectively. Similar results regarding the reduction of leaf area with increasing concentration of saline solution were observed in previous studies on *A. canadensis* and *Tagetes* sp. (marigold) (Sun et al., 2018; Wu et al., 2016a).

Number of shoots and flowers. Saline solution irrigation affected the number of shoots, but there were no interactive effects between salt treatment and Sego Supreme™ plant species. With increasing EC levels, the number of shoots of *C. fruticosa* and *T. acaulis* decreased linearly [r^2 = 0.88 (P = 0.0003) and r^2 = 0.84 (P = 0.004), respectively], but the number of shoots of *E. septentrionale* decreased quadratically [R^2 = 0.98 (P = 0.0007)] (Fig. 2D). Similarly, Sun et al. (2015) reported that the number of shoots of *M. arboreus*, *S. leucantha*, and *V. ×hybrida* decreased significantly when irrigated with saline solution at an EC of 10.0 dS·m^{-1} for 8 weeks, whereas the number of shoots of *S. farinacea* was not reduced. Although the number of shoots of *T. scaposa* tended to decrease with saline solution irrigation at an EC of 10.0 dS·m^{-1} for 5 weeks but still was not significant (Wu et al., 2016b). In addition, *A. barnebyi* had no significant reduction in the number of shoots in this study.

In the same way, increasing salt levels of the irrigation water reduced the number of flowers linearly in *E. septentrionale* and *T. acaulis* [r^2 = 0.74 (P = 0.0001) and r^2 = 0.97 (P = 0.001), respectively] (Fig. 3A). Compared with the control, the number of flowers of *E. septentrionale* and *T. acaulis* decreased by 46.5% and 50.3%, respectively, when irrigated with saline solution at an EC of 10.0 dS·m^{-1}. According to the study by Wu et al. (2016b), *T. scaposa* had a 50% reduction in the number of flowers when irrigated weekly with saline solution at an EC of 5.0 dS·m^{-1} for 5 weeks. High salinity levels can affect the development of flower buds, which fail to open or grow in flowering woody shrubs or trees (Azza-Mazher et al., 2007). *Aquilegia barnebyi* and *C. fruticosa* did not produce any flowers during the experimental period. However, lower numbers of shoots may help to predict a reduction in the number of flowers with increasing salt concentrations in the irrigation water. Razmjoo and Aslani (2018) actually observed a 20% reduction in the number of flowers of *C. ispahanica* when irrigated with saline solution at an EC of 16.3 dS·m^{-1}.

Shoot dry weight. Shoot dry weight decreased quadratically for *A. barnebyi* and *E. septentrionale* [R^2 = 0.95 (P = 0.001) and R^2 = 0.99 (P = 0.002), respectively] with increasing EC levels, but decreased linearly for *C. fruticosa* and *T. acaulis* [r^2 = 0.92 (P < 0.0001) and r^2 = 0.88 (P = 0.0017), respectively] (Fig. 3B). Compared with controls, the shoot dry weight of *A. barnebyi*,

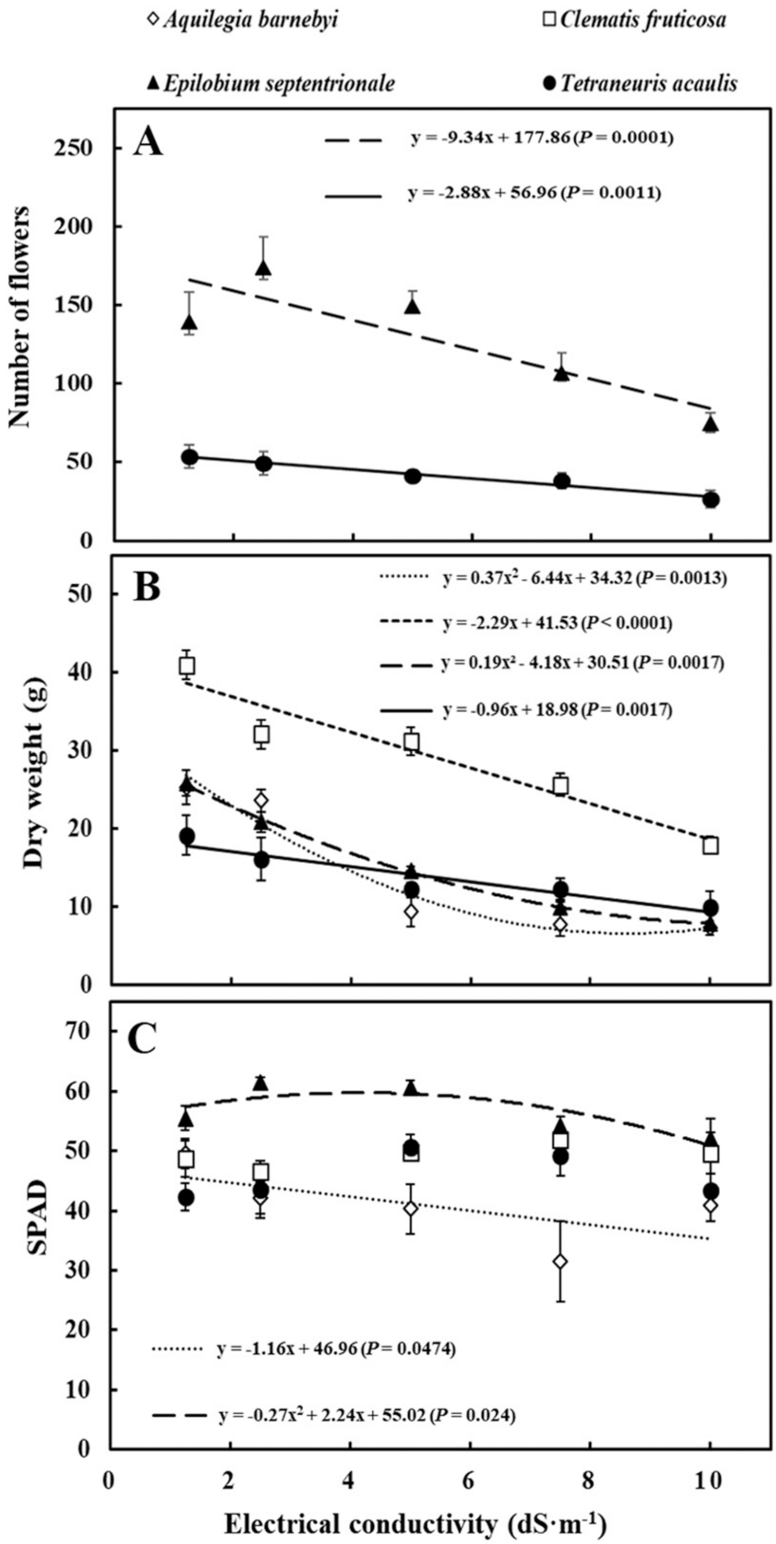

Fig. 3. Number of flowers (**A**), shoot dry weight (**B**), and relative chlorophyll content [soil-plant analysis development (SPAD)] (**C**) of Sego Supreme™ plants after irrigating with a nutrient solution at an electrical conductivity of 1.25 dS·m^{-1} or a saline solution at an EC of 2.5, 5.0, 7.5, or 10.0 dS·m^{-1}. A nutrient solution at an EC of 1.25 dS·m^{-1} was prepared by adding 0.8 g·L^{-1} 15N–2.2P–12.5K water-soluble fertilizer (Peters Excel 15–5–15 Cal-Mag Special) to tap water. Sodium chloride (NaCl) and calcium chloride ($CaCl_2$) were added to the nutrient solution to make the saline solutions. No flowers were observed in *A. barnebyi* and *C. fruticosa* during the experimental period. *Clematis fruticosa* and *E. septentrionale* had no significant reduction in SPAD readings with increasing EC levels. Vertical bars represent standard errors of six measurements.

C. fruticosa, *E. septentrionale*, and *T. acaulis* irrigated with saline solution at an EC of 10.0 dS·m^{-1} decreased by 71.3%, 56.3%, 69.7%, and 48.1%, respectively. Elevated salinity has been reported to reduce plant shoot dry weight in various ornamental plants such as *A. canadensis*, aster perennials, *E. laxum*, and rose rootstocks (Hadi et al., 2014; Niu et al., 2008; Wu et al., 2016a, 2016b). This is a consequence of salinity-induced water deficit, resulting in stunted growth and reduced leaf size described earlier.

Relative chlorophyll content (SPAD). Elevated salinity affected the SPAD readings of the four Sego Supreme™ plants with varying responses among species. *Clematis fruticosa* and *T. acaulis* had similar SPAD readings among different EC levels, which indicated that increased salinity level did not affect their relative chlorophyll content, but the SPAD readings decreased linearly [r^2 = 0.42 (P = 0.05)] for *A. barnebyi* and quadratically [R^2 = 0.75 (P = 0.02)] for *E. septentrionale* (Fig. 3C). Saline solution at an EC of 10.0 dS·m^{-1} decreased the SPAD readings of *A. barnebyi* and *E. septentrionale* by 17.7% and 6.2%, respectively. Similarly, Sun et al. (2015) documented that the SPAD readings of *M. arboreus*, *R. brittoniana*, and *V. ×hybrida* did not change, but *S. farinacea* and *S. leucantha* decreased significantly when irrigated weekly with saline solution at an EC of 10.0 dS·m^{-1} for 8 weeks. In a study of rose rootstocks by Niu et al. (2008), *Rosa ×fortuniana* had greater SPAD readings compared with *R. ×multiflora* and *R. ×odorata* when irrigated with saline solution at an EC of 6.0 dS·m^{-1} for 15 weeks. These results consistently indicated that salinity stress negatively affected the chlorophyll content and the effect is dependent on plant species.

Mineral analyses. Sodium and Cl^- concentrations in the leaf tissue of Sego Supreme™ plants were significantly affected by both elevated salinity and plant species (Table 2). Sodium concentration of *A. barnebyi*, *C. fruticosa*, *E. septentrionale*, and *T. acaulis* increased linearly [all r^2 values ≥0.93 (all P values < 0.0001)] with increasing salt levels in the irrigation water. Compared with the respective control, *A. barnebyi*, *C. fruticosa*, *E. septentrionale*, and *T. acaulis* had 4 to 7 times increased Na^+ concentration when irrigated with saline solution at an EC of 2.5 dS·m^{-1}. Similarly, the Na^+ concentrations were further increased by 17, 37, and 53 times for *A. barnebyi*; 13, 25, and 33 times for *C. fruticosa*; 9, 26, and 38 times for *E. septentrionale*; and 8, 18, and 35 times for *T. acaulis* when plants irrigated with saline solutions at ECs of 5.0, 7.5, and 10.0 dS·m^{-1}, respectively. Chloride concentration of *A. barnebyi*, *C. fruticosa*, *E. septentrionale*, and *T. acaulis* increased linearly [all r^2 values ≥0.93 (all P values < 0.0001)] with increasing amount of salts in the irrigation water (Table 2). Compared with the respective control, the Cl^- concentration increased by 7, 24, 41, and 48 times for *A. barnebyi*, 6, 10, 13, and 16 times for *C. fruticosa*, 2, 6, 15, and 17 times for *E. septentrionale*, and 4, 5, 14, and 17 times for *T. acaulis* when plants were irrigated with saline solutions at ECs of 2.5, 5.0, 7.5, and 10.0 dS·m^{-1}, respectively. Wu et al. (2016b) observed that *T. scaposa* irrigated weekly

Table 2. Leaf ion concentrations of Sego Supreme™ plants irrigated with a nutrient solution at an electrical conductivity (EC) of 1.25 $dS \cdot m^{-1}$ or a saline solution at an EC of 2.5, 5.0, 7.5, or 10.0 $dS \cdot m^{-1}$ for 8 weeks.[z]

		Ion concn ($mg \cdot g^{-1}$ dry wt)			
Species	EC	Na^+	Cl^-	Ca^{2+}	K^+
Aquilegia barnebyi	1.25	0.2	1.2	8.5	24.7
	2.5	0.9	9.6	10.4	25.2
	5.0	2.8	29.2	17.6	24.9
	7.5	5.9	49.6	20.9	24.5
	10.0	8.5	56.9	23.4	22.8
	L[y]	***[y]	***	***	NS
	Q	NS	*	*	NS
Clematis fruticosa	1.25	0.1	1.1	13.1	15.0
	2.5	0.7	8.2	13.7	16.7
	5.0	1.3	11.9	13.1	15.4
	7.5	2.4	15.6	16.3	12.8
	10.0	3.2	18.9	18.0	11.2
	L	***	***	**	*
	Q	NS	*	NS	NS
Epilobium septentrionale	1.25	0.2	1.6	11.0	12.9
	2.5	0.9	5.2	11.8	11.9
	5.0	1.5	11.1	12.2	10.3
	7.5	4.2	24.8	17.9	9.3
	10.0	5.9	28.8	19.9	8.7
	L	***	***	***	***
	Q	NS	NS	NS	NS
Tetraneuris acaulis	1.25	0.4	3.9	13.9	32.1
	2.5	2.0	17.9	16.5	27.0
	5.0	3.2	24.0	16.4	24.5
	7.5	6.9	57.2	22.9	18.8
	10.0	13.1	71.6	29.2	18.0
	L	***	***	***	***
	Q	NS	NS	NS	NS

[z]A nutrient solution at an EC of 1.25 $dS \cdot m^{-1}$ was prepared by adding 0.8 $g \cdot L^{-1}$ 15 N–2.2 P–12.5 K water-soluble fertilizer (Peters Excel 15–5–15 Cal-Mag Special) to tap water. Sodium chloride (NaCl) and calcium chloride ($CaCl_2$) were added to the nutrient solution to make the saline solution.

[y]For linear (L) or quadratic (Q) trend analyses, NS, *, **, *** denote nonsignificant or significant at $P <$ 0.05, 0.01, or 0.001, respectively.

with saline solutions at an EC of 5.0 and 10.0 $dS \cdot m^{-1}$ for 5 weeks had 29 and 58 times more Na^+ concentration and 9 and 21 times more Cl^- concentration in leaves than the control. These results indicate that *T. scaposa* accumulated much more Na^+ concentration in the leaves than *T. acaulis* in the study, but similar Cl^- concentrations are observed in the leaves of both species. In another similar study, *A. canadensis* also accumulated a significant amount of Na^+ and Cl^- ions in the leaves (Wu et al., 2016a), which were 6 and 35 times more for saline irrigation water at an EC of 5.0 $dS \cdot m^{-1}$, respectively, and 70 and 62 times more at an EC of 10.0 $dS \cdot m^{-1}$, respectively, compared with their respective control.

Sodium and Cl^- are common ions present in reclaimed water and cause leaf injury to plants (Wahome et al., 2001). Generally, salt-tolerant ornamental plants accumulate less Na^+ and Cl^- ions in their leaves when compared with salt-sensitive plants (Wu et al., 2016a). In this study, Na^+ and Cl^- concentrations significantly increased in *A. barnebyi* compared with the other three species, which is in line with the lower visual quality of *A. barnebyi* with increasing salt levels in the irrigation water. There was a significant correlation of visual score to Na^+ [$r = -0.94$ ($P < 0.0001$)] and Cl^- [$r = -0.96$ ($P < 0.0001$)] concentration. These results point out that, like *A. canadensis*, *A. barnebyi* has low tolerance of Na^+ and Cl^- accumulation and poor potential to exclude these ions from plant tissue. On the other hand, some plants can tolerate accumulated Na^+ and Cl^- in shoot tissue (Munns and Tester, 2008). In this study, *T. acaulis* and *C. fruticosa* accumulated high levels of Na^+ and Cl^- ions in the leaves and still had good visual quality. These results indicate that *T. acaulis* and *C. fruticosa* can tolerate high accumulation of Na^+ and Cl^- ions in the tissue. Krupenikov (1946) also reported that *Clematis orientalis* (chinese clematis) was tolerant over 2.0% of total salts, which is calculated on the basis of solid residue. Similarly, Wu et al. (2016a) indicated that *Scabiosa columbaria* (butterfly blue), *Lobelia cardinalis* (cardinal flower), *A. canadensis*, and *Cuphea hyssopifolia* (mexican false heather) plants had severe foliar salt damage with increasing Na^+ and Cl^- concentration in shoots, whereas *Dicliptera suberecta* (Mexican hummingbird bush) and *Cestrum diurnum* × *C. nocturnum* 'Orange Peel' (orange peel jessamine) plants had the ability to tolerate high Na^+ and Cl^- accumulation without a reduction in visual quality.

Calcium concentration in the leaf tissue was affected by both salt treatment and plant species interactively (Table 2). The Ca^{2+} concentration in the leaves of *A. barnebyi*, *C. fruticosa*, *E. septentrionale*, and *T. acaulis* increased linearly [$r^2 = 0.96$ ($P < 0.0001$), $r^2 = 0.83$ ($P = 0.008$), $r^2 = 0.91$ ($P < 0.0001$), and $r^2 = 0.91$ ($P = 0.0004$), respectively] with elevated salt levels in the irrigation water. There was only a slight increase in the Ca^{2+} concentration compared with the increment in Na^+ and Cl^- concentration, although $CaCl_2$ was used to prepare the saline solution. Compared with the control, leaf Ca^{2+} concentration of *A. barnebyi* irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ increased by 175.3%, which was the greatest increment observed among all tested plants in all treatments. In addition, there was a significant correlation of visual score and Ca^{2+} concentration of *A. barnebyi* [$r = -0.89$ ($P < 0.0001$)]. Nazrul-Islam (1986) reported that *Epilobium hirsutum* (great willowherb) grows well in calcareous soil and is susceptible to lower calcium level. Similarly, in the present study calcium level may not be sufficient for *E. septentrionale* at higher EC levels and they showed more reduction in shoot dry weight when compared with *C. fruticosa* and *T. acaulis*. Moreover, plant requirements for Ca^{2+} ions increase with increasing concentration of salts in the root zone. However, Ca^{2+} transport and mobility to growing parts of plant is reduced by Na^+ dominated salinity stress (Grattan and Grieve, 1999).

Increasing salt concentration in the irrigation water linearly decreased the amount of K^+ ions in the leaves of *C. fruticosa*, *E. septentrionale*, and *T. acaulis* (Table 2). However, elevated salinity did not affect the K^+ concentration of *A. barnebyi*. Compared with the control, the leaf K^+ concentration of *C. fruticosa, E. septentrionale*, and *T. acaulis* irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ declined by 25.1%, 32.7%, and 43.8%, respectively. When plants are exposed to salinity stress induced by high NaCl levels, Na^+ accumulation increases and causes a reduction in K^+ concentration (Hasegawa et al., 2000). Similar observations are made on *Chrysactinia mexicana* (damianita), *T. scaposa*, *Santolina chamaecyparissus* (lavender cotton), *Leucanthemum* ×*superbum* (Shasta daisy), and *Viguiera stenoloba* (skeletonleaf goldeneye) (Wu et al., 2016b), *S. columbaria* and *C. diurnum* × *C. nocturnum* 'Orange Peel', but not for *A. Canadensis* (Wu et al., 2016a).

In summary, Sego Supreme™ plants had different responses to saline water irrigation. *Clematis fruticosa* and *T. acaulis* were the most tolerant perennials with the least reduction in visual score and growth when irrigated with saline solution. *Epilobium septentrionale* was moderately tolerant, and *A. barnebyi* was the least tolerant perennial to saline solution irrigation with the most reduced visual quality and growth while showing the greatest increase in leaf Na^+ and Cl^- concentrations. These results are helpful for selecting salt tolerant Sego Supreme™ plant species for use in the landscape where reclaimed water is used for irrigation.

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